# PBPK Modelling

The uptake and disposition of chemicals (originally drugs and pharmaceuticals) by living organisms, most notably humans, has long been one of the primary concerns of pharmacological research. The study of the dispersion of chemicals in animal bodies entails the field of pharmacokinetics -- although, depending on the 'type' of chemical studied, it has also been called toxicokinetics, and biokinetics. For drug research, two parameters of the disposition of a drug are of special interest, viz. the residence time of a compound in the body, most often reported as its half life, and the (peak or average, or both) target concentration, the amount of the drug that actually reaches the pharmacodynamic target site and is thus in principle available for therapeutic (inter)action. Because of the limited resources available for both detailed, time-course in-vivo concentration studies, and for the computational effort needed, pharmacokinetic investigators traditionally described the pharmacokinetics of a compound with data-based compartmental models -- they generally measure the time-course of the compound's concentration in a 'central' compartment, normally blood, hypothesize a number of peripheral compartments of unknown size and chemical disposition, and fit a n-exponential model to the blood concentration vs. time data, n being the number of hypothesized compartments (Klaassen).

While this approach generally works well enough if one has the data needed to fit the model to, some researchers clearly weren't overly pleased with the apparent physiological meaninglessness of the compartments and compound rate constants that constituted the n-exponential model. So as early as 1937, Teorell described a modelling approach that did not start from more or less 'arbitrary' data, but started with a functional, but parsimonious physiological description of a living body, dividing it into key tissue groups, with connecting blood flows providing inter-tissue transport, and membrane permeabilities and tissue partition coefficients describing the distribution of a compound over the different physiological compartments. In these original applications of physiologically based modelling one of the major bottlenecks was the fact that in order to use the model, the set of linked differential equations actually had to be solved either analytically, or by 'manually' performed numerical approximation techniques. Consequently this approach evolved only slowly during the next 40-odd years, until during the 70s, major advances in computer hardware and software made it possible for the first time to actually solve the large sets of dependent differential equations needed to adequately describe a basic physiologically-based pharmacokinetic model within a reasonable amount of time 'from the desktop'.

So the basic difference between a classical pharmacokinetic model and a physiologically-based pharmacokinetic model is as follows: In the classical model not only the (compound) rate constants and concentrations, but also the actual layout of the model (the 'topology' of the model; the number and size of the compartments and the interconnections between them) is dependent on the experimental data (and thus on the chemical under study, the setup of the experiment, the route of exposure, and the dosing regimen, a.o.). In the physiologically-based model on the other hand, most, if not all, of the model topology, as well as a lot of the rate constants and other chemical-dependent parameters can be determined a priori. The actual model topology can be determined by taking into consideration the animal under study and its physiology, the specific target for the chemical (drug) under study and its physiological location, and the specific metabolic and eliminatory pathways that are known for a drug, e.g., for a drug that is known not to pass the so-called blood/brain barrier, a separate brain compartment is not needed for an adequate, parsimonious PBPK model. The chemical parameters needed for the adequate description of its pharmacokinetics can also be determined a priori; blood/air and tissue/blood partition coefficients can be measured in-vitro, by a number of different techniques (vial-headspace equilibration for volatiles (Sato, Gargas), dialysis equilibration

(Lin) or Solid Phase Microextraction equilibration (De Jongh) for non-volatiles). Metabolic rate constants, for either Michaelis-Menten saturable degradation or for linear nth-order (normally first order) kinetics can also be measured in-vitro, with e.g. liver homogenates for liver based metabolism (Wilkinson). Then, when the model is complete, it can be checked against actual experimental time-course data for the chemical and animal under study, and if the correspondence is found lacking, some of the parameters, within physiological and chemical constraints, can be adjusted to improve the model. Such a PBPK model has two major advantages over a classical model:

- 1] Since it is based on the actual physiology of an animal, it not only empirically describes the concentration time-course in the compartment that was actually measured, but will also predict the concentration time-course in all the other recognized compartments, notably the compartment holding the active target for a drug. The model can in principle even be refined to the point where it will accurately predict the concentration of a pharmacon at the specific target molecule.
- 2] Since it explicitly contains the physiology description of an animal, and the pertinent chemical parameters of the compound under interest, it opens up the way of actual cross-species (or intra-species cross-lifestage) or cross-compound predictions. It is explicitly this extrapolation possibility that makes PBPK (and/or PBPD) modelling such a promising tool in predictive toxicology.

So what does a basic PBPK model look like? A basic mammalian model, assuming inhalatory exposure to a volatile organic compound is made up of the following compartments: lungs (the alveolar space part of them), lung blood, where the compound first enters the body proper, fat (adipose) tissue (this normally acts as a storage/sink compartmentfor lipophilic compounds, with back-delivery), richly perfused tissue (kidney, intestines,brain, liver (this is normally separated out from the rest of the richly perfused tissue because it is the compartment where the metabolism is normally located) and poorly perfused tissue (mainly muscle and bone). Then there is the blood, normally divided in arterial blood, leaving the lungs laden with chemical, and entering the tissues, where it will redistribute it, and venous blood, leaving the tissues, depleted with chemical, then returning to the lungs. The overall blood flow is described by cardiac output and by the fractional blood flows entering the separate compartments. For other exposure routes additional 'compartments' can be defined, such as a skin compartment for dermal uptake, a bulk bolus 'compartment' for I.P. injection, or a gut compartment for uptake through food. Additional elimination compartments, besides exhalation via the lungs, can be kidney, for urinary exrection, liver for biliary excretion, or gut for direct desorption into fecal mass. See Figure 1 for a schematic overview of a basic PBPK model.

The model is linked together mathematically by means of so-called mass-balance equations, that describe the movement of compound from blood to tissue, or vice-versa, by considering the difference in concentration (or amount) of chemical in the arterial blood entering, and the venous blood exiting a tissue compartment (Andersen):

$$\frac{dA_i}{dt} = Q_i C_a - Q_i (C_i / P_{b,i})$$

This equation indicates that the rate of change in amount of chemical in compartment i is a function of the blood flow to the compartment Qi times the concentration in the afferent blood Ca ('what's coming in'), minus the blood flow from the compartment (again Qi) times the concentration in the efferent blood Ci/Pb,i , defined as the concentration in the tissue over the tissue/blood partition coefficient

('what's going out'); this description assumes that all compartments are internally well mixed, and that the efferent blood is in equilibrium with the tissue (Cvl = Ci/Pb,i). This type of basic model is called a flow-limited model. If the assumption of tissue/blood equilibrium isn't met for one or more compartments, we get a so-called diffusion-limited model, where two differential equations are needed for each compartment, one for the rate of change in the tissue proper, and one for the rate of change in the tissue blood.

Figure to be placed yet...

figure 1. Schematic overview of the main compartments and blood and substance flows in a basic PBPK model. Qalv = alveolar ventilation; Cinh = concentration of compound in inhaled air; Calv = concentration of compound in alveolar air; Qt = cardiac output, or total blood flow rate; Cart is concentration of compound in arterial blood; Qm, Qf, Qr and Ql, are the blood flow rates to individual tissues (Qf + Qm + Qr + Ql = Qt); Cvf, Cvm, Cvr and Cvl are the concentrations of the compound in the efferent (venous) blood exiting the respective compartments; Cven = the concentration of compound in the venous blood entering the lungs (Cvf + Cvm + Cvr + Cvl = Cven); Vmax and Km are Michaelis-Menten kinetic constants. Figure adapted from Ramsey and Andersen

The major asset of PBPK/PD modelling for mixture toxicity work is that, in principle, modelling the pharmacokinetics of a set of more than one compound is mathematically the same as modelling the kinetics of a single compound. Fortunately, the current state of computational technology, both in hardware and in software, is such that models of such a complexity can easily be solved, even from the desktop, if a powerful enough computer (high end desktop system, e.g. employing Intel P5 or Motorola PowerPC hardware, or a workstation type of machine) is used. In the simplest case, one assumes that the individual compounds do not interact at all. This leads to a model that is nothing more than a repetitious extension of a single compound model, where the only mixture toxicity integration has to be performed as the possible adding of the multiple amounts at a target site. Of course this assumption, while often quite useful in practice, will be in violation of theoretical considerations in almost all instances. Fortunately, a well-defined PBPK/PD model can incorporate known interactions between compounds at any of a number of levels, viz.: 1] at the level of transport kinetics, where they could compete for free sites on transport proteins, 2] at the level of metabolism and elimination, where they could either competitively or non-competitively inhibit each others turnover by one or more enzyme systems, and 3] at the level of the pharmacodynamics, where they could competitively inhibit each others binding to an active site at a target enzyme, or where one compound could display non-competitive inhibition or stimulation of the action of another compound, or even act as an antagonist. In some cases it could even be that interactions of different compounds at disjoint target sites ultimately have an interactive (synergistic or antagonistic) toxic effect. If known, all of these types of interactions can in principle be provided for in a PBPK/PD model.

# Software:

About 95% of all PBPK Modelling is done with ACSL (for Advanced Continuous Simulation Language) from Mitchell & Gauthier, which is a programming environment geared specifically to the definition of dynamic systems through sets of linked differential equations and the automagical numerical solution of same. As you can see from my Curriculum Vitae, I spent 6 months learning PBPK Modelling at CSU, Ft. Collins, CO, USA, where I used ACSL for model definition. However, back home at RITOX I use a Macintosh, for which no ACSL version is available; since I DO have MATLAB on my machine, and are fairly familiar with its capabilities, I adapted MATLAB, together with the new ODEsuite from the Mathworks (for the numerical solution of (linked) differential

equations) for my PBPK work. A sample MATLAB PBPK program is given here for reference:

# cch.m

```
%PROGRAM: CCH.CSL PHYSIOLOGICAL MODEL
%          'CLOSED CHAMBER MODEL'
%         ' -- USING STEADY-STATE APPROXIMATION'
%          'CSU PB-PK COURSE AUGUST 1994'
%         'PREPARED BY HARVEY CLEWELL'
%
%Adapted to MATLAB by Henk Verhaar
%AT = y(:,1);                                              %Amount in tissues (mg)
%CT = AT/VT;
%AF = y(:,2);                                              %Amount in fat tissue (mg)
%CF = AF/VF;
%AL = y(:,3);                                              %Amount in liver tissue (mg)
%CL = AL/VL;
%AMS = y(:,4);                                             %Amount metabolized by saturable pa
%AML = y(:,5);                                             %Amount metabolized by linear pathw
%AM = AMS + AML;
%ACH = y(:,6);                                             %Chamber amount (mg)
%CCH = ACH/VCH;
%CCPPM = CCH*24450./MW;                                    %Concentration in chamber (ppm)
%RVB = QL*CL/PL+QT*CT/PT+QF*CF/PF;        %Amount in venous blood
%CV = RVB/QC;                                              %Concentration in venous blood
%CA = (QC*CV+QP*CCH)/(QC+QP/PB);          %concentration in arterial blood
%CX = CA/PB;                                               %Exhaled concentration
%TMASS = NMICE*(AF+AL+AT+AM)+ACH;         %Mass balance

%INITIAL

%Physiological Parameters ***********************************'

global BW QCC QPC QLC QFC VLC VFC

 BW = 0.04;                                                %Body weight (kg)
QCC = 18.;                                        %Cardiac output (L/hr-1kg)
QPC = 30.;                                        %Alveolar ventilation (L/hr-1kg)
QLC = 0.25;                                               %Fractional blood flow to liver
QFC = 0.05;                                               %Fractional blood flow to fat
VLC = 0.04;                                               %Fraction liver tissue
VFC = 0.05;                                               %Fraction fat tissue

%Chemical specific parameters ********************************'

global PB PL PF PT MW VMAXC KM KFC

   PB = 8.3;                                      %Blood/air partition coefficient
   PL = 1.7;                                      %Liver/blood partition coefficient
   PF = 14.5;                                     %Fat/blood partition coefficient
   PT = 1.0;                                      %Slowly perfused tissue/blood partition
   MW = 85.;                                      %Molecular weight (g/mol)
VMAXC = 14.;                                      %Capacity of saturable metabolism (mg/hr)
   KM = 0.5;                                      %Affinity of saturable metabolism (mg/L)
  KFC = 2.;                                               %Linear metabolism rate (/hr)

%Calculated parameters

global VF VL VT QC QP QL QF QT VMAX KF

      VF = VFC*BW;                        %Volume of fat - L
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```
      VL = VLC*BW;                          %Liver volume - L
      VT = 0.91*BW-VL-VF;       %Tissue volume - l
      QC = QCC*BW^0.74;                     %Cardiac output - L/hr
      QP = QPC*BW^0.74;                     %Alveolar ventilation - L/hr
      QL = QLC*QC;                          %Liver blood flow - L/hr
      QF = QFC*QC;                          %Fat blood flow - L/hr
      QT = QC-QF-QL;            %Tissue blood flow L/hr
    VMAX = VMAXC*BW^0.7;        %Saturable capacity - mg/hr
      KF = KFC/BW^0.3;                      %Linear pathway

%Parameters for simulation of experiment**********************'

global NMICE VCHC CONC VCH ACH0

NMICE = 11.;                                        %Number of mice in chamber
 VCHC = 9.1;                                        %Volume of chamber (L)
 CONC = 1500.;                                      %Initial concentration in chamber (ppm)

 VCH = VCHC - NMICE*BW;                 %Adjusted chamber volume
ACH0 = VCH*CONC*MW/24450.;      %Initial amount in chamber (mg)

%Timing commands

 TSTOP = 2.;                                        %Length of experiment (hrs)
POINTS = 240.;                                      %Number of points in plot
DOSES  = 12;                                        %Number of consecutive doses
%Exposure Definitions

 CINT  = TSTOP/POINTS;                  %Communication interval

%Integration (initial) values

period = [0 TSTOP];

TIJD = [];
AT0 = 0.;
AT  = [];
AF0 = 0.;
AF  = [];
AL0 = 0.;
AL  = [];
AMS0 = 0.;
AMS  = [];
AML0 = 0.;
AML  =[];
ACH0 = VCH*CONC*MW/24450.;
ACH  =[];
y0 = [AT0;AF0;AL0;AMS0;AML0;ACH0];

%End of initial

%DYNAMIC

%DERIVATIVE

for i = 1:DOSES

[t,y] = ode15s('cch_der',period,y0);

TIJD = [TIJD;t];                                    %Time vector
AT = [AT;y(:,1)];                                   %Amount in tissues (mg)
CT = AT/VT;
AF = [AF;y(:,2)];                                   %Amount in fat tissue (mg)
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```
CF = AF/VF;
AL = [AL;y(:,3)];                                   %Amount in liver tissue (mg)
CL = AL/VL;
AMS = [AMS;y(:,4)];                                 %Amount metabolized by saturable pathway (mg)
AML = [AML;y(:,5)];                                 %Amount metabolized by linear pathway (mg)
AM = AMS + AML;
ACH = [ACH;y(:,6)];                                 %Chamber amount (mg)
CCH = ACH/VCH;
CCPPM = CCH*24450./MW;                              %Concentration in chamber (ppm)
RVB = QL*CL/PL+QT*CT/PT+QF*CF/PF;
                                                                    %Amount in venous blood
CV = RVB/QC;                                                %Concentration in venous blood
CA = (QC*CV+QP*CCH)/(QC+QP/PB);
                                                                    %concentration in arteria
CX = CA/PB;                                                     %Exhaled concentration
TMASS = NMICE*(AF+AL+AT+AM)+ACH;
                                                                    %Mass balance
period = period + TSTOP;
laatst = length(TIJD);
y0 = [AT(laatst);AF(laatst);AL(laatst);...
            AMS(laatst);AML(laatst);ACH(laatst)+ACH0];

end

%End of derivative
%End of dynamic
%End of program
```

# cch_der.m

```
function ydot = cch_der(t,y)

global BW QCC QPC QLC QFC VLC VFC
global PB PL PF PT MW VMAXC KM KFC
global VF VL VT QC QP QL QF QT VMAX KF
global NMICE VCHC CONC VCH ACH0

ydot = zeros(size(y));


AT = y(1);                                          %AT = Amount in tissues (mg)
CT = AT/VT;
AF = y(2);                                          %AF = Amount in fat tissue (mg)
CF = AF/VF;
AL = y(3);                                          %Amount in liver tissue (mg)
CL = AL/VL;
AMS = y(4);                                                 %Amount metabolized by saturable pathway (mg)
AML = y(5);                                                 %Amount metabolized by linear pathway (mg)
AM = AMS + AML;
ACH = y(6);                                                 %Chamber amount (mg)

%experimental section
%END of experimental section

CCH = ACH/VCH;
CCPPM = CCH*24450./MW;                      %Concentration in chamber (ppm)
RVB = QL*CL/PL+QT*CT/PT+QF*CF/PF;
                                                                    %Amount in venous blood
CV = RVB/QC;                                            %Concentration in venous blood
CA = (QC*CV+QP*CCH)/(QC+QP/PB);
                                                                    %concentration in arterial blood
CX = CA/PB;                                                 %Exhaled concentration
```

```
TMASS = NMICE*(AF+AL+AT+AM)+ACH;
                                                        %Mass balance


ydot(1) = QT*(CA-CT/PT);
ydot(2) = QF*(CA-CF/PF);
ydot(3) = QL*(CA-CL/PL) - VMAX*CL/PL/(KM+CL/PL) - KF*VL*CL/PL;% - RAM
ydot(4) = VMAX*CL/PL/(KM+CL/PL);
ydot(5) = KF*VL*CL/PL;
ydot(6) = QP*(CX-CCH)*NMICE;

%END of function
```

If you're familiar with ACSL, you'll probably notice that the code above is really adapted ACSL code, insstead of de nove MATLAB code.

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